

A HISTOCHEMICAL STUDY OF THE DERMO-EPIDERMAL MEMBRANE IN COWHIDE*

SUMMARY

The dermo-epidermal membrane was demonstrated to have fibrous elements, a few scattered cellular elements, and amorphous elements. The fiber elements were reticular fibers (the principal fiber) and elastin. A few scattered fibroblasts were demonstrated close to the area of the corium minor. The amorphous material is suggested to be mucoprotein and/or glycoprotein.

INTRODUCTION

The dermo-epidermal membrane of skin in rats (3), mice (3), humans (8 (p. 304), 14) and cowhide (2) has been reported as periodic acid-Schiff (PAS) positive, and containing reticular fibers. The positive PAS reaction of the basement membrane has been variously attributed to the presence of a lipo-gluco-protein complex (1), glycoprotein (3), chondroitin sulfate B bound to protein (9), chondroitin sulfate B and hyaluronic acid (12), and mucopolysaccharides (13). This investigation was undertaken to further investigate the PAS positiveness of the dermo-epidermal membrane and the presence of previously unmentioned compounds, fibers, and other structures. The dermo-epidermal membrane will be referred to as the basement membrane hereafter.

MATERIALS AND METHODS

Cowhide was used as a source of material for this study. The hide was obtained immediately after slaughter, allowed to cool (1¼ hours), washed, trimmed, and stored in a deep freeze (-40°C). As the need arose, hide was removed

* A report of work done under contract no. 12-14-100-2623 (73) with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract was conducted through the Lowell Technological Institute Research Foundation and was supervised by the Eastern Utilization Research and Development Division of the Agricultural Research Service.

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from the deep freeze, thawed, and cut into blocks for purposes of fixation and sectioning. Fixation was accomplished by the routine use of formal saline. Additional fixatives as Lillie's alcoholic lead nitrate (8 (p. 42)) for mucoproteins and mucopolysaccharides, and alcohol formalin and Carnoy's for glycogen staining were also employed.

Fixed frozen section were cut 6 to 8 μ thick on a Spencer sliding microtome with a Histofreeze attachment.

The fixed sections were stained for the following components:

a. Elastin: orcein (6 (p. 256)).

b. Reticular fibers: a modification of the Bielschowsky and Maresch methods (8 (p. 343)).

c. Collagen: Van Gieson's picro acid fuchsin, 100 mg per 100 ml picric acid solution, plus 0.25 ml of concentrated HCl (8 (p. 346)).

d. Triglycerides: acetic carbol Sudan III (4) and acetylated Sudan black B (8 (p. 304)) the procedure was modified to include the use of Celite as a filtering aid, the acetylated product which adhered to the Celite was removed by washing with acetone.

e. Metachromasia: 0.05% thionin at pH 4 for 30 minutes (8 (p. 286)).

f. Cholesterol and its esters: the Schultz methods (8 (p. 317)).

g. Phospholipids: dichromate-fixed sections extracted with acetone at room temperature (6 (p. 165)) and fixed unextracted sections were stained with acid hematein.

h. PAS-positive material: two staining methods were used, an alcoholic periodic acid-Schiff reagent with a sulfite rinse and picric acid counterstain (2), plus Pearse's orange G Hotchkiss alcoholic periodic acid technique (11 (p. 831)). These methods were used in parallel.

i. Vicinal hydroxyls (carbohydrates): Bauer's method (5 (p. 59)).

j. Glycogen: iodine technique (8 (p. 276)).

k. Acid mucopolysaccharides: Steedman's Alcian blue as modified by Pearse (11 (p. 838)) and Hale's dialyzed iron technique (7), the dialyzed iron used was obtained from the British Drug House Ltd., Poole, England.

l. Basophilic substances: Dempsey and Singer's methylene blue extinction (11 (p. 836)).

TABLE 1
Staining Reactions of the Amorphous Material in the Basement Membrane

Method	Amorphous Material of Basement Membrane
Tyrosine (protein).....	+
Sudan black B.....	—
PAS.....	+
Diastase.....	Fast
Metachromasia (γ).....	—
Metachromasia (β).....	+
Methylene blue extinction technique.....	Above pH 4
Aleian blue (pH 2).....	—
Hale (7).....	—
Hyaluronidase.....	Fast
Pepsin and trypsin plus PAS.....	—
Phospholipids.....	—

m. Protein (tyrosine): the Millon reaction according to Bensley and Gersh (11 (p. 791)).

The fixed sections were also treated with the enzymes hyaluronidase, pepsin, and trypsin, and then stained by PAS (11). The hyaluronidase treatments were carried out in the following manner. Sections were fixed in formol saline, washed in distilled water, and incubated at 37.5°C in well slides with a hyaluronidase buffer solution (25 mg hyaluronidase (Armour) dissolved in 100 ml 0.1 *M* Veronal-acetate buffer, (pH 6.77) for 15 hours; the sections were then washed and stained. The trypsin treatments were performed as follows. Sections were fixed in formol saline, washed in distilled water, and incubated in well slides at 37.5°C for 15 minutes to 3 hours at a pH 8.9 using phosphate buffer (0.05 *M*) containing 1 mg of trypsin (L814, Matheson, Coleman and Bell) per ml. The sections were then washed and stained. The pepsin treatments were carried out as follows. Sections were fixed in formol saline, washed in distilled water, and incubated in well slides for 1 to 3 hours at 37.5°C in 0.12 *N* HCl pH 1.6 solution containing 2 mg crystalline pepsin (380204 Matheson, Coleman and Bell) per ml. The sections were then washed and stained.

RESULTS

The basement membrane was found to have three components, amorphous material, fibers (elastic and reticular), and a few fibroblasts which appear to penetrate the region from the corium. The principal fibrous elements were reticular fibers with a few scattered elastin fibers penetrating the basement membrane from the

corium minor. The interwoven fibers were imbedded in a matrix of amorphous material.

The amorphous material was found to be moderately PAS positive and strongly protein positive (tyrosine), negative for triglycerides, steroids, and their esters, glycogen, mucopolysaccharides, and phospholipids. The methylene blue extinction test was negative at pH below 4 and positive at pH 4.1 and above. The β -metachromatic reaction was positive using buffered thionin. In sections pretreated with pepsin or trypsin, basement membranes lost their PAS reaction; in sections predigested with hyaluronidase, they retained their positive PAS reaction.

The occurrence of elastin and fibroblasts in addition to reticular fibers in the basement membrane was not unexpected, for the dividing line between the basement membrane and corium minor is not sharp and clean-cut. Therefore, one could reasonably expect some components of the corium minor (elastin and fibroblasts) to occur to some extent in the basement membrane area.

The amorphous material of the basement membrane was previously described as PAS positive. In order to ascertain the nature of the PAS-positive reaction of the basement membrane, the scheme of Pearse (11 (pp. 236-239)) was utilized. Pearse (11 (p. 235)) has stated that compounds such as polysaccharides, mucopolysaccharides, glycoproteins, glycolipids, unsaturated lipids, and phospholipids gave PAS-positive reactions. The results of the staining experiments were tabulated and compared with the reactions as cited in Pearse's scheme. Comparison of Table 1 and Pearse's scheme suggest that the PAS-positive material could be mucopolysaccharide and/or glycoprotein. Additional support for this statement was the negative PAS reaction obtained when the sections were pretreated with trypsin or pepsin and then stained by the PAS technique.

An attempt to characterize further the amorphous material as mucopolysaccharide or glycoprotein, or a combination of both, was made. Meyer (10) has defined mucoprotein as a complex (protein and hexosamine) which has more than 4% hexosamine bound to the protein, whereas glycoprotein has less than 4% hexosamine similarly bound. The Bauer technique (8 (pp. 120-122)), a sire of the PAS staining procedure, was then used. In Bauer's technique, chromic acid is used as an oxidant before placing in the Schiff reagent.

The positive Bauer reaction is of interest, in that the basement membrane was also colored by this less sensitive reaction, as well as by the periodic acid-Schiff reaction. Such an observation suggests the presence of a relatively higher concentration of vicinal hydroxyls, *i.e.*, carbohydrate, than is seen with a negative Bauer reaction. Hence, on the basis of the preceding experimental results, it is suggested that a muco- or glycoprotein, or a combination of both, makes up the bulk of the amorphous material in the basement membrane.

Acknowledgment: The technical assistance of N. Bissaillon and D. Reed is gratefully acknowledged.

REFERENCES

1. DUPRE, A. Histochemical studies of the human skin. I. The dermo-epidermal junction. *Ann. Derm. Syph.* **80**: 263-274, 1953.
2. EVERETT, A., AND CORDON C. The use of salt to assist enzymatic unharing of fresh hides with histochemical observations. *J. Amer. Leather Chem. Ass.* **53**: 548-564, 1958.
3. GERSH, I., AND CATCHPOLE, H. The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth. *Amer. J. Anat.* **85**: 457-521, 1949.
4. GLICK, D. *Techniques of Histo- and Cytochemistry*. Interscience Publishers, Inc., New York, 1949.
5. GOMORI, G. *Microscopic Histochemistry*. Univ. Chicago Press, Chicago, 1952.
6. GUYER, M. *Animal Micrology*. Univ. Chicago Press, Chicago, 1953.
7. HALE, C. Histochemical demonstration of acid polysaccharides in animal tissues, *Nature*, **157**: 802, 1946.
8. LILLIE, R. *Histopathologic Technic and Practical Histochemistry*. Blakiston Co., New York, 1954.
9. MEYER, K. *Cold Spring Harbor Symp. Quant. Biol.* **6**: 91-98, 1938.
10. MEYER, K. Chemistry of connective tissue. In *Trans. First Conference on Connective Tissue*, pp. 88-101. Josiah Macy Foundation, New York, 1950.
11. PEARSE, A. *Histochemistry, Theoretical and Applied*, 2nd ed. Little, Brown, & Co., Boston, 1960.
12. RIZZOLE, C. The nature and distribution of mucopolysaccharides of the skin. *Dermatologica* **103**: 349-361, 1951.
13. ROBB-SMITH, A. The nature of reticulin. In *Trans. Third Conference on Connective Tissue*, pp. 92-116. Josiah Macy Foundation, New York, 1952.
14. STOUGHTON, R., AND WELLS, G. A histochemical study on polysaccharides in normal and diseased skins. *J. Invest. Derm.* **15**: 37-51, 1950.